（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K868-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader (575-595 nm)

Detection range：3.12-250 U/mL

Elabscience® Chitinase Activity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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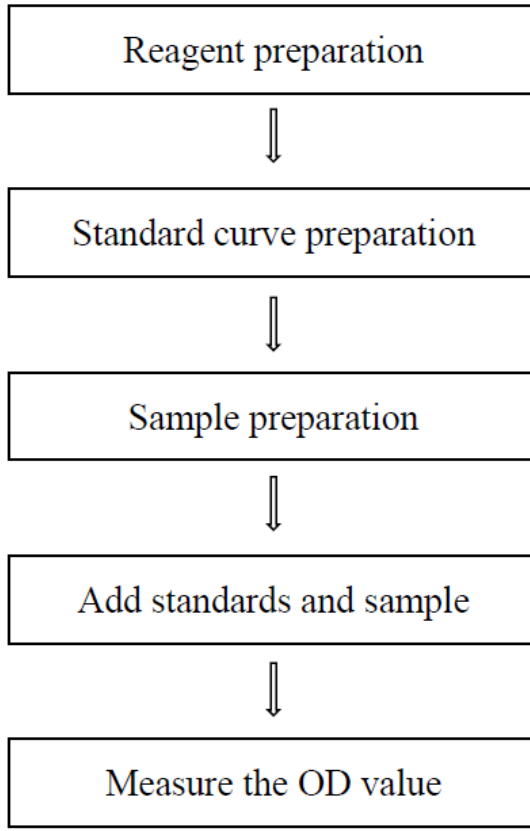
Assay summary



Intended use

This kit can be used to detect the chitinase activity in shell tissue and fungus samples.

Detection principle

Chitinase mainly exists in the shell of crustaceans such as shrimp, crabs, insects, organs of mollusks and cell walls of fungus. Chitinase can catalyze the hydrolysis of chitinase and has the function of resisting fungus infection, so it has become the research focus of anti-fungus diseases.

This kit can calculate the activity of chitinase by measuring the OD value change at 585 nm in unit time of chitinase catalyzed chitinase hydrolysis products.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	30 mL ×1 vial	2-8℃, 12months
Reagent 2	Buffer Solution	15 mL ×1 vial	2-8℃, 12months
Reagent 3	Mareix Solution	12 mL ×1 vial	2-8℃, 12months
Reagent 4	Saline Solution	5 mL ×1 vial	2-8℃, 12months
Reagent 5	Chromogenic Agent	Powder×2 vials	2-8℃, 12months
Reagent 6	Chromogenic Diluent Solution	70 mL ×1 vial	2-8℃, 12months
Reagent 7	Standard	Powder×1 vial	2-8℃, 12months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (575-595 nm, optimum wavelength: 585 nm), Incubator (37 ℃), Water bath

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of working solution：

Dissolve one vial of chromogenic agent with 32 mL of chromogenic diluent solution, mix well to dissolve. Store at 2-8 ℃ for 4 weeks protected from light (If the chromogenic diluent solution crystallizes, incubate at 37℃ to dissolve).

③ The preparation of 5 mg/mL standard solution：

Dissolve one vial of standard with 1 mL of buffer solution, mix well to dissolve. Store at 2-8 ℃ for 4 weeks protected from light.

④ The preparation of 100 μg/mL standard solution：

Before testing, please prepare sufficient 100 μg/mL standard solution according to the test wells. For example, prepare 1000 μL of 100 μg/mL standard solution (mix well 20 μL of 5 mg/mL standard solution and 980 μL of buffer solution). Store at 2-8 ℃ for 3 days protected from light.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μg/mL standard solution with buffer solution diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 60, 80, 100 μg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μg/mL)	**0**	**10**	**20**	**30**	**40**	**60**	**80**	**100**
100 μg/mL Standard (μL)	0	20	40	60	80	120	160	200
Buffer solution (μL)	200	180	160	140	120	80	40	0

Sample preparation

Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 50 mg tissue in 450 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Fungus sample:

① Harvest the number of fungus needed for each assay (initial recommendation 1×10^6 fungus).

② Wash fungus with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 fungus in 200 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 20 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

The key points of the assay

① The mareix solution is a suspension and mix well before use..

② The saline solution is a saturated solution, which will have crystal precipitation at low temperature (2-8 ℃) conditions and can be heated at 60 ℃ to dissolve.

③ After the reaction, the reaction solution should be added to the plate well as soon as possible for detection

Operating steps

Enzymatic reaction

① Sample tube: Add 80 μL of sample to the corresponding tubes.
Control tube: Add 80 μL of sample to the corresponding tubes.

② Add 40 μL of buffer solution to each tube.

③ Add 80 μL of mareix solution to each tube.

④ Mix fully and incubate at 37 ℃ for 60 min protected from light.

⑤ Boiling water bath for 5 min. Centrifuge at 10000×g for 5 min at 25℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

Chromogenic reaction

① Standard tube: Add 100 μL of standard solution with different concentrations into the corresponding tubes.
Sample tube: Add 100 μL of sample supernatant which was collected in sample tube after the reaction of the enzyme reaction step into the corresponding tubes.
Control tube: Add 100 μL of sample supernatant which was collected in control tube after the reaction of the enzyme reaction step into the corresponding tubes.

② Add 20 μL of saline solution to each tube.

③ Control tube: Mix fully and stand at 25 ℃ for 5 min.
Standard tube: Mix well, boil water for 5 min and cool to room temperature.

Sample tube: Mix well, boil water for 5 min and cool to room temperature.

④ Add 300 μL of working solution to each tube.

⑤ Incubate at 37℃ for 20 min protected from light. Collect 200 μL solution to corresponding wells. Measure the OD value of each well at 585 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue or fungus samples:

Definition: The amount of enzyme in 1 mg sample protein per 1 h that produce 1 μg product at 37 ℃ is defined as 1 unit.

$$\text{chitinase activity (U/mgprot)} = (\Delta A - b) \div a \div V_1 \times V_2 \div C_{pr} \times f \div T$$

[Note]

ΔA: $\Delta A = A_{sample} - A_{control}$.

V_1: The volume of sample added to the enzymatic reaction system, 80 μL.

V_2: The volume of enzymatic reaction system, 200 μL.

C_{pr}：The concentration of protein in sample, mgprot/mL.

f: Dilution factor of sample before test.

T: Reaction time, 1 h.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three shrimp head shell tissue samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	50.00	100.00	200.00
%CV	5.0	4.1	3.2

Inter-assay Precision

Three shrimp head shell samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	50.00	100.00	200.00
%CV	8.6	4.5	3.2

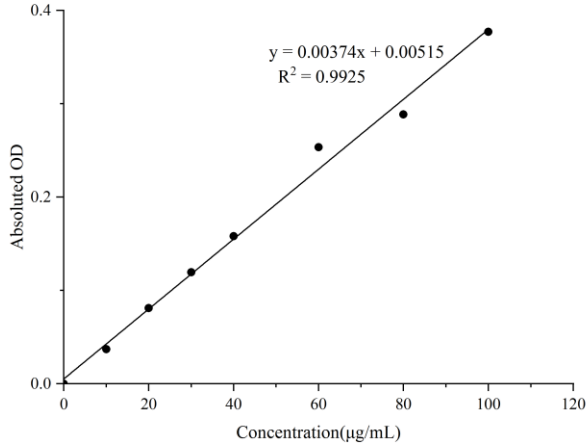
Sensitivity

The analytical sensitivity of the assay is 3.12 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μg/mL)	0	10	20	30	40	60	80	100
OD value	0.081	0.118	0.158	0.200	0.241	0.306	0.372	0.455
	0.078	0.115	0.163	0.198	0.234	0.360	0.364	0.458
Average OD	0.080	0.117	0.161	0.199	0.238	0.333	0.368	0.457
Absoluted OD	0.000	0.037	0.081	0.120	0.158	0.254	0.289	0.377



Appendix II Example Analysis

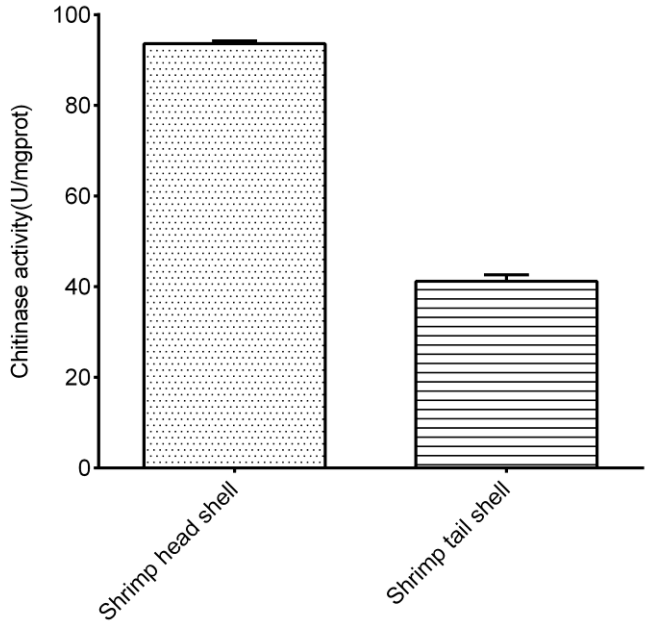
Example analysis:

Take 80 μL of 10% shrimp head shell tissue homogenization which dilute for 2 times and carry the assay according to the operation steps. The results are as follows: standard curve: y = 0.00374 x + 0.00515. The OD value of sample is 0.374, the OD value of control is 0.086, the concentration of protein is 4.06 mgprot/mL, and the calculation result is:

chitinase activity (U/mgprot) = (0.374 – 0.086 - 0.00515) ÷0.00374 ÷4.06 ÷80 ×200 ×2 ÷1

= 93.13 U/mgprot

Detect 10% shrimp head shell tissue homogenization (the concentration of protein is 4.06 mgprot/mL, dilute for 2 times) and 10% shrimp tail shell tissue homogenization (the concentration of protein is 3.80 mgprot/mL), according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.